MITOCHONDRIA AND OTHER CYTOPLASMIC STRUCTURES IN THE SPERMATOGENESIS OF PASSALUS CORNUTUS.

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A. INTRODUCTION.

In course of my study on the spermatogenesis of *Passalus cornutus* (one of the Lacunid beetles), the mitochondria and other cytoplasmic inclusions came to my attention. Although I was primarily interested in the study of the nuclear changes with especial reference to synapsis, it seemed desirable to make a study of the cytoplasmic structures above mentioned, since they showed so clearly in my preparations. I shall therefore reserve for a later report a description of the nuclear changes involved in the course of spermatogenesis.

I am glad to acknowledge my indebtedness to Prof. E. G. Conklin, of Princeton University, under whose guidance and criticism this work has been pursued. I also wish to thank Mr. Milton P. Hunter, of Westtown, Pa., from whom I received the material used in this study.

B. MATERIAL AND TECHNIQUE.

There are two pairs of more or less bulb-shaped testes situated in the posterior part of the abdomen. These were dissected out in Ringer's solution and immediately fixed in one of the following fixation fluids: Hermann's, Flemming's strong solution, and Benda's modification of Flemming's fluid. Iron hæmatoxylin followed by either Lichtgrün, erythrosin or Bordeaux red was the stain most used, especially when the fixations were with Hermann's or Flemming's fluids. The Benda crystal violet-alizarin staining method was employed on the material fixed in the Benda fluid. On the whole, material fixed in strong Flemming's fluid (three or four hours) and then stained in iron hæmatoxylin followed by one of the counterstains previously men-

tioned, was the most satisfactory. In Hermann and Benda material there is a tendency, especially in the spermatocytes, for the mitochondria to agglutinate in dense irregular masses.

C. Observations.

(*a*) *Chromosomes.*

Although not primarily concerned with the chromosomes in the present report, it may be of some interest to at least mention these in a general way. I have as yet been unable to find spermatogonial plates of sufficient clearness to make accurate drawings, but all counts indicate that the chromosome number is 26, including an unequal pair. This has been confirmed by a study of the ovaries in which all dividing follicle cells show 26 chromosomes and these can be arranged in equal pairs (Fig. 3). The metaphase plate of the first spermatocyte (Figs. 12, 33) shows 13 bivalent chromosomes, one of which represents the unequal (sex) pair. The elements of the sex pair separate in the first maturation division and divide equationally in the second division.

(*b*) *Mitochondria.*

I shall not attempt to review the vast literature which has grown up bearing on the subject of the mitochondria. Duesberg ('11) has given a rather complete review of the literature, so that it will not be necessary to do so here. I shall, however, discuss the works of others insofar as these may be related to my observations on *Passalus*.

1. *Spermatogonia.*—In the primary spermatogonia, which are situated at the blind end of the testes, I have been unable to find conclusive evidence of the presence of mitochondria (Fig. 1). The cytoplasm is usually of a homogeneous, non-reticular and finely granular structure. These granules do not stain in anything but the plasma stain, and therefore cannot be considered as mitochondria. There are usually present, in the cytoplasm, several deeply staining bodies (hæmatoxylin) which are perhaps similar to the chromatoid corpuscles of other workers. It is possible that these may really be of a mitochondrial nature, but their subsequent fate cannot in any way be related to the mitochondria of the later stages. Lewis and Robertson (1916) in

their study of the living cells of *Chorthippus* (Orthoptera) found that mitochondria were present in the primary spermatogonia in the form of granular threads; but there is no evidence for such structures in *Passalus*.

The secondary spermatogonia are arranged in cysts in the form of a rosette, and are generally pyramidal in shape. Both nuclear and cytoplasmic volume is noticeably smaller in these than in the primary gonia. The cytoplasm always shows a marked affinity for the hæmatoxylin and this is due to the presence of numerous mitochondrial granules (Figs. 4, 23) which are scattered diffusely throughout the cytoplasm. Payne (1917) found granular mitochondria in the spermatogonia of *Gryllotalpa*, while Duesberg (1910) figures similar structures in *Blaps*. On the contrary Duesberg describes the mitochondria of the gonia of *Blatta* as being present in the form of threads (chondrioconts). Payne, Schäfer ('07) and others have found that the mitochondria of the spermatogonia are localized at the inner ends of the cells (*i. e.*, the end bordering on the cyst cavity). As I have before stated, in *Passalus* the mitochondria are diffusely spread and often are actually absent from the inner ends of the cells. Montgomery (1911) was unable to find evidence of "indubitable mitochondria" in the spermatogonia of *Euschistus*, although he found granules which he considered to be disintegrated idiozome material.

Fig. 5 shows a degenerating spermatogonium. It is noticed that the cytoplasm is much more deeply staining and the mitochondrial granules are much larger. The chromatin of the nucleus is concentrated into one or two karyosomes. The increase in the size of the mitochondrial granules is probably due to an agglutination of the smaller normal ones. Cowdry (1916) in his excellent review on the functional significance of mitochondria, has called attention to the relations of mitochondria in pathological tissues. He mentions the work of Scott who found that in fatty degeneration of the pancreas there was an agglutination of the mitochondria. It is quite possible that the degeneration of cells which is so common in insect spermatogenesis is of the fatty degeneration type. A study of the behavior of the mitochondria in degenerating sperm cells may throw some light

on their rôle in normal processes. This is, however, beyond the scope of the present work.

The spermatogonial cysts which are in mitotic activity, stand out very clearly in contrast with the resting cysts. This is because of their lighter staining capacity; whether this is in turn due to the partial disappearance of the mitochondria, could not be ascertained (Figs. 2, 24). Buchner (1909) found that in *Gryllotalpa vulgaris* the mitochondria disappear during or just before cell-division. There are three possible explanations for the partial loss of mitochondrial structure during mitotic activity; (*a*) at this time the cytoplasmic volume is much greater and hence the mitochondria are more diffusely spread; (*b*) the dissolution of the nuclear membrane sets free a large amount of karyolymph which perhaps dilutes the cytoplasm and obscures the mitochondria; (*c*) the mitochondria may dissolve or become chemically changed so that they are no longer recognizable as such. At any rate, they soon appear in great numbers after cell-division, so that their partial disappearance was apparent and not real.

2. *Spermatocytes.*—In the spermatocytes at the beginning of the growth period, the mitochondria are still in the form of diffusely spread granules. There is a noticeable increase in their numbers as the growth period progresses, which plainly indicates that new ones are being formed (Fig. 6). Usually a denser perinuclear zone of cytoplasm can be seen during the growth period; the significance of this zone will be discussed later.

As the late prophase approaches, the cytoplasm becomes filled with numerous delicate threads deeply staining in hæmatoxylin. These are the filar mitochondria (chondrioconts) and they first appear at this time, although there is a slight indication of delicate granular threads in some of the earlier stages. The origin of these threads could not be traced, but it is quite likely that they are genetically related to the granules of the preceding stages. In the first spermatocyte the mitochondria appear diffusely spread when material has been fixed in Flemming's fluid (Figs. 7, 8, 25, 25*a*). In Benda and Hermann material the mitochondria agglomerate in dense irregular masses, still showing their filar nature (Figs. 9, 26). The threads tend to

lie with their length extended in the direction of the main axis of the cell. Sections of the cell taken at right angles to its longitudinal axis show the mitochondria on end view (Fig. 25*a*). As the nuclear membrane dissolves and the first maturation spindle is forming (Figs. 10, 29), the mitochondria are usually localized at one side of the spindle. When the spindle has fully formed, the mitochondria begin to envelop it at first from one side (Fig. 11) and later entirely surround the outer spindle fibers, being equally distributed along their lengths (Figs. 13, 30, 31). By the time the metaphase has been reached, the spindle has been entirely surrounded by mitochondria. Figures 12, 32, 33, 34 represent cross-sections at different levels of the first maturation spindle at metaphase, showing clearly the relations of spindle and mitochondria. As is best seen in the anaphase (Figs. 13, 35) they do not entirely reach to the two poles of the spindle, so that there is a short uncovered part near the centrosomes.

The question arises as to the method of movement of the mitochondria in order that they establish this relation with the spindle; is it an active or a passive movement? Many observers on living cells have noted that the mitochondria are generally of a vibratile or motile nature. According to Lewis and Lewis (1914) the mitochondria are never at rest; "a single mitochondrium sometimes twists and turns rapidly as though attached at one end, like the lashing of a flagellum, then suddenly moves off to another position in the cytoplasm as though some tension had been released" (p. 332). If it is true that they possess the power of active movement, it may be possible in this way to explain their movement to the spindle. Lewis and Robertson (1916) state that "the mitochondria migrate from the two masses of mitochondrial granules and elongate towards the poles of the spindle," etc. (p. 108). Whether they mean by this an active or a passive migration is not clear from their account. It seems highly improbable that the movement is active. Some observers have supposed that their movement is influenced by the centrosomes, but there is no conclusive evidence that this is the case. It is more likely that the movement of the mitochondria to their position on the spindle is due to the cytoplasmic

movements during metakinesis which Conklin has shown, plays such an important rôle in the localization of cytoplasmic substances in the egg.

There is no evidence from my observations on *Passalus* that the mitochondria divide autonomously, as has been maintained by some workers. As the cell constriction continues through the equator of the spindle (Figs. 14, 35), the mitochondria are divided by this constriction and the daughter cells (second spermatocytes) receive approximately equal amounts of mitochondria. The mitochondrial masses then move a short distance toward the poles; this is evidently caused by a further elongation of the spindle. Montgomery (1911) has described a similar method of division in *Euschistus*. The fact that mitochondria are found in equal amounts in daughter cells is supposed by some to favor the view that they divide autonomously; but it is well known that yolk and similar substances are often found in equal amounts in daughter cells, yet no one maintains that yolk granules divide autonomously. Fauré-Fremiet (1911) describes autonomous division in the mitochondria of the Protozoa, and Wilke (1912) does likewise for the spermatocytes of *Hydrometra*. Payne (1917) does not incline strongly to the view that the mitochondria are divided by the cell constriction, while Lewis and Robertson (1916) do not clearly state whether their division in *Chorthippus* is autonomous or passive.

Following the first maturation division, there is a short interkinesis during which the second maturation spindle is formed (Figs. 16, 36). At this time the mitochondria are lying at one side in a rather dense mass which shows a lighter central portion through which the spindle of the previous division passed. The behavior of the mitochondria during the second maturation division is precisely the same as in the first division (Figs. 17, 37). They again surround the spindle peripherally and are divided by the equatorial constriction. As a result of this division both spermatids receive approximately equal amounts of mitochondria (Fig. 18).

The mitochondrial mass contained in the spermatid gradually becomes more compact and henceforth may be designated as the Nebenkern. It stains intensely with the basic stains and

often shows a granular structure. It often shows a lighter central portion which represents the position the spindle had occupied (Fig. 20*d*). Soon after the Nebenkern has become a spherical body, it begins to show a peripheral lighter area which is of a vacuolar nature (Fig. 19). As the spermatid elongates the Nebenkern becomes elongated and the vacuolization of the peripheral layer becomes more marked (Fig. 20, *a*, *b*). It becomes divided into two halves and is later pierced by the axial filament (Figs. 21, 39). As the axial filament grows out, the Nebenkern continues to elongate forming a sheath about it. The material of the Nebenkern thus extends from the centrosome for a considerable distance along the axial filament (Fig. 22). Fig. 20, *c* is that of a cross-section through the tail of a spermatid showing the two halves of the Nebenkern on each side of the axial filament. Whether or not the Nebenkern sheath extends to the free end of the axial filament could not be determined. As the transformation continues, the Nebenkern becomes more lightly staining, and all evidences of its mitochondrial nature are lost.

3. *Discussion.*—I have been unable to obtain any definite and conclusive evidence as to the manner in which the mitochondria arise. There can be no doubt that they increase in numbers during the growth period, and the question arises: to what influence do the mitochondria owe their origin? This has been and still is a much-debated cytological question, and much depends on the final solution of the problem.

Meves, Bouin, Duesberg and others have always maintained that the mitochondria are persistent and self-perpetuating structures, much as the chromosomes of the nucleus are at present regarded. On the other hand, Goldschmidt and his pupils, Buchner, Jordan, Wildman and others have derived the mitochondria from the nucleus, and have thus maintained that they are akin to the chromidia of Hertwig. Vejdovský has traced their origin from the sphere material, while Montgomery in *Euschistus* concludes that they are probably derived from the idiozome or the nucleus, or by a "joint action" of both. In their studies on the living sperm cells of *Chorthippus*, Lewis and Robertson are unable to find any evidence that the mitochondria

are derived from the nucleus. It is thus evident that the facts are conflicting on all sides. If it is true, as I have previously stated (p. 408), that the mitochondria are not present in the primary spermatogonia of *Passalus*, then we cannot maintain that they are persistent and self-perpetuating. I am, however, not entirely convinced that they are absent in the primary spermatogonia and shall await fresh material for a study of the living cells.

It is possible that the increase in number of the mitochondria during the growth period is due to a simple growth and division of those already present. I cannot find any evidence for this in my material. In nearly all the growth stages of the first spermatocytes, there is present a denser and more deeply staining perinuclear zone. Schäfer (1907) has figured similar conditions in *Dytiscus*, while Voivnov (1903) shows it more strikingly in *Cybister* and calls it the "zona interna." During the maturation divisions, the "zona interna" surrounds the spindle peripherally and forms the Nebenkern of the spermatid. As Duesberg (1910) has pointed out, it is quite evident from this behavior that the "zona interna" is really of a mitochondrial nature. Giardina (1904) has studied the oöcytes of a number of forms (*Periplaneta*, *Stenobothrus*, *Gryllus*, *Mantis*, etc.) and figures well-defined perinuclear zones usually of a granular nature. Whether this zone is formed by a diffusion of chromatin in the form of a solution out of the nucleus into the surrounding cytoplasm or whether it arises in situ in the cytoplasm by interaction with the nucleus, is a question which Giardina discusses at considerable length. He concludes in favor of the latter view. Payne (1917) has described a similar perinuclear zone in the oöcytes of *Gryllotalpa*, but he definitely states that this consists of mitochondria. As the oöcyte grows, the mitochondria migrate centrifugally into the cytoplasm. Vejdovský (1911–12) shows the mitochondria arranged in a definite perinuclear zone in the spermatocytes of *Diestramena*. It thus seems quite probable that the perinuclear zones described by Giardina are really of a mitochondrial nature. The occurrence of this zone in such close connection with the nucleus and especially during a period when the mitochondria are certainly increasing in number, is

highly indicative of an interaction between nucleus and cytoplasm. The relation of this zone to the mitochondria seems to be a strong argument, at least, that it is the locus of mitochondria formation.[1]

The association of the spindle and the mitochondria has led to some confusion in regard to the origin of the Nebenkern, so that some workers have ascribed its origin to the spindle remains of the second maturation division. Arnold (1908) comes to this conclusion in his study of *Hydrophilus*, as does Baumgartner in *Gryllus*. Voivnov, as previously mentioned, derived the Nebenkern from his "zona interna" and the peripheral spindle fibers. Munson (1906) in his work on *Papilio* also derives the Nebenkern from the spindle remains, but his observations are interesting since they clearly show that he has confused these with the mitochondria. According to his view, it is only the "outer, granular" mantle fibers of the spindle which take part in the formation of the Nebenkern. It is evident, in light of the recent work, that the "outer, granular" mantle fibers are really mitochondria. That he actually saw the mitochondria and misinterpreted them, is evident from the following quotation: "Often a few scattered chromatin segments are found scattered along the spindle fibers, or else drawn out into stainable threads parallel with the spindle fibers" (p. 91).

(*c*) *Spindle Derivatives.*

When one attempts to review the literature on the subject of spindle derivatives and their histories, one is immediately confronted with a maze of conflicting observations and interpretations, to say nothing of a nomenclature which is almost hopelessly confused. The names mitosome, idiozome, attractionsphere, centrosphere, astrosphere, Nebenkern (of older workers), etc., are all examples of the existing confusion, and should caution us against hasty interpretations of such structures. Meves (1899) states that he first applied the term "idiozome" to those compact bodies in the spermatogonia and spermatocytes which

[1] It is interesting to note in this connection that the material of the yellow crescent in the ascidian egg has been shown by Conklin to be found at various times in a perinuclear position; and Duesberg (1913) has shown that this zone is extremely rich in mitochondria.

surround the central corpuscles (centrosomes). By a disintegration of the idiozome, the centrosomes are set free and then take part in the next cell-division. In the rat, however, Meves found that there is no disintegration of the idiozome, but that the centrosomes wander out, leaving the idiozome intact. As division progresses, the latter dissolves and disappears. There is no essential difference between the "attraction-sphere" of Van Beneden, the "centrosphere" of Strasburger and the "astrosphere" of Fol and Boveri; and so far as I have been able to ascertain, there is no fundamental difference between these last named structures and the "idiozome" of Meves. One thing is clear,—that these structures all refer to the achromatic substance of the spindle situated at the poles and usually enclosing the central corpuscles. To avoid any possible misuse of these terms I shall employ the non-committal term "sphere" to denote this portion of the spindle. With regard to the remains of the spindle proper, there is less confusion of terms, and I shall use the term "mitosome" or "spindle remains" to designate this structure.

1. *Spermatogonia.*—The spermatogonia in *Passalus* in mitotic activity stand out very sharply in contrast with those in the resting condition not only because of their lighter staining capacity (as mentioned on p. 410), but also because they tend to become round in outline and the cell walls become more sharply defined. This is indicative of an internal pressure which Reinke (1900) calls the "mitotic pressure." As the gonial anaphase progresses, a well-defined cell-plate makes its appearance and stains deeply in hæmatoxylin (Figs. 2, 24). As the telophase advances, the spindle and cell-plate become more compact, the former taking the acid stains and the latter taking the basic stains. Fig. 4 shows several resting cells from a spermatogonial cyst with the spindle remains (mitosome) running from cell to cell. Very often the spindle remains from each division become so connected that they form sort of bond between all the cells in a cyst. Hegner (1914) has described a similar condition in the spermatogonial cysts of *Leptinotarsa decemlineata.* In this case, however, he found that material fixed in Carnoy's fluid always showed the spindle remains taking the basic stain.

Günthert (1910) has described similar results in the differentiation of nurse cells and oöcytes in *Dytiscus* and Govaerts (1913) has done likewise in *Carabus*. In *Passalus*, it is only in the region of the cell-plate that the spindle takes the basic stain. In cross-section the spindle remains appear as plasmasome-like bodies in the cytoplasm. Often there may be seen deeply staining granules adhering to the surface of such bodies; these I take to be portions of the deeply-staining cell-plate.

2. *Spermatocytes.*—The spindle remains of the last spermatogonial division (together with the cell-plate) persist into the spermatocyte and remain very conspicuous throughout the entire growth period (Figs. 6, 8, 27). Every spermatocyte exhibits these spindle remains, either in their original positions connecting cells or else in the form of plasmasome-like bodies lying free in the cytoplasm. Similar conditions have been shown by Voivnov in *Cybister* and by Munson in *Papilio*. In *Gryllotalpa*, Payne states that "there is no indication that a sphere or the spindle and astral fibers persist after cell-division." In his figure B, plate 1, he shows two plasmasome-like bodies present in the cytoplasm of a spermatocyte during the early growth period. As to the origin of these bodies, he is uncertain, but he is not ready to admit that they may be idiozome material. I agree that it would be rather speculative to assign the term "idiozome" to these bodies, but in *Passalus* it is quite clear that similar bodies are derived from the spindle. I am not able to establish the presence of a definite "idiozome" or sphere material in the spermatocytes of *Passalus* and just where the centrosomes lie hidden during the growth period is a difficult matter to determine. Since the cells are so filled with mitochondria at this time, it seems almost impossible to definitely locate them. The centrosomes are first discernible when the first maturation spindle makes its appearance. From the long persistence of the spindle remains of the last spermatogonial division, it may be concluded that its substance must be of some inert, resistant material. Munson (1906, p. 90) makes the following remarks on *Papilio:*

"The resistance of the maturation spindles to reagents is remarkable. In studying the living dividing cells on the slide,

I have seen the cytoplasm gradually disintegrate, become vacuolated, and disappear while the spindles remain as perfect as ever. In testing the effects of reagents, too, I have been able to dissolve practically the whole of the cytoplasm of all the cells of the cyst, while the spindles remained, showing a system of connected spindles throughout the whole cyst."

There are other structures related to the spermatocytes which are of a more or less problematical nature. In Figs. 8 and 28 there will be noticed a large vacuole in each of the spermatocytes; these are to be found in nearly every spermatocyte during the late prophases and thus far I have been unable to find them in the earlier stages. The vacuoles are filled with a homogeneous fluid (perhaps a gel) which takes the plasma stain but lightly. Sometimes there may be several vacuoles in a single cell. In cysts of spermatocytes having such vacuoles, one often finds in the cyst cavities round or oval bodies of a granular nature (Fig. 8) resembling very much the cytoplasm of the spermatocytes. Furthermore, in such cysts the sides of the spermatocytes bordering on the cyst cavity are of an irregular outline and often distinct pseudopod-like projections of the cytoplasm are given off. It is therefore highly probable that the small bodies found free in the cyst cavity have arisen from the pseudopod-like projections or buddings from the cytoplasm of the spermatocytes. What the significance of either the vacuoles mentioned above, or of the casting off of portions of the cytoplasm may be, I am unprepared to state. The presence of vacuoles and pseudopods at the same time may indicate that the vacuoles are cast out by means of setting free the pseudopods. But this view is at present untenable, for I have never actually seen vacuoles within the pseudopods; furthermore the contents of the vacuoles are always of a homogeneous appearance, while the cast off parts of the cytoplasm are granular as described above. Voivnov has found in *Cybister* that vacuoles make their appearance in the late prophases of the spermatocytes. These differ in appearances, at least, from those found in *Passalus* in that they contain definite bodies as inclusions. Voivnov also finds that they are later cast out into the cyst cavity. He considers these to be portions of the sphere (idiozome) which are under-

going disintegration. In support of this view, he finds that they appear at a time when the centrosomes are first set free and often they may be found in close spatial relations to the centrosomes.

Whether or not the vacuoles and the cast-off portions of the cytoplasm in *Passalus* correspond to the conditions described by Voivnov in *Cybister* I am at present unable to say. The origin of the vacuoles and their relation to the cytoplasmic buddings can be best studied in the living material, and I shall leave the question undecided here for lack of evidence.

3. *Maturation Divisions and the Spermatid.*—The spindles developed for both maturation divisions are relatively very large, both in actual dimensions and in number of spindle fibers. Correlated with the large size of the spindles is the relatively large nucleus, containing little chromatin but a large amount of karyolymph. The view of Conklin and others, that the spindle grows at the expense of the karyolymph is certainly substantiated in the case of *Passalus*. In both maturation divisions cell-plates are developed which are very much smaller than those found in the spermatogonia (Figs. 14, 8), and the remains of the spindles persist for a considerable time after division. In the anaphase of the second maturation division, the centrosomes are still distinguishable lying close to the chromatin masses at the poles. In the telophase of this division they are found lying on the nuclear membrane. It is because of this position on the nuclear membrane that they are not easily detected, but careful searching and focusing will show their undoubted presence (Fig. 18). Thus in the spermatid, the centrosome is still to be seen closely attached to the nuclear membrane; it gradually shifts its position until it comes to lie between the nucleus and the Nebenkern. Usually there is a precocious growth of the axial filament (Fig. 19) before the centrosome arrives at its ultimate position. At this time, the centrosome appears double, while the axial filament grows out between the two halves of the Nebenkern and becomes associated with the latter in the formation of the tail, as shown before (p. 413). As transformation continues (Figs. 21, 22), the centrosomes become more and more closely associated with the nucleus, until in the older stages they are scarcely

distinguishable from the head of the spermatozoön, and hence there is no well-defined middle-piece.

In the youngest spermatid there is always present a cytoplasmic body of a refringent nature which takes the plasma stain (Figs. 19, 38). One portion of it is more compact, while the other part is in the form of a vacuole containing a deeply staining (hæmatoxylin) body. This structure is undoubtedly a spindle derivative, but whether it represents the sphere material or the mitosome I am unable to state. As to the origin of the deeply staining body contained within it, I am equally unable to explain. It may possibly represent a portion of the cell-plate which has become detached from the cell wall and has become encompassed in the remains of the spindle.[1] In older spermatids a compact portion of the spindle derivative is found lying near the nucleus in that part of the spermatid which is destined to give rise to the head end of the spermatozoön (Fig. 21). In later stages, as the nucleus becomes laterally compressed, this structure is transformed into the acrosome. The remainder of the spindle derivative is sloughed off into the tail of the spermatozoön and gradually disappears.

4. *Discussion.*—As has been previously stated, the axial filament in *Passalus* arises in connection with the centrosome. Munson (1906) has expressed an entirely different view concerning its origin in *Papilio*. His view is that the axial filament represents a much compressed portion of the cytoreticulum and has no relation to the centrosome. Accordingly, he finds that in the stages of development of the axial filament occasionally three or four filaments may be present in a single spermatid, but these finally unite into a single thread. Paulmier (1899) has described double and quadruple spermatids in which two or four axial filaments were present, but each was connected to a centrosome. Munson's view arises from the fact that he has assigned a wholly different function to the centrosome. His figures of the spermatids of *Papilio* show spindle derivatives each containing a deeply staining body which he interprets as

[1] Duesberg (1908) figures a similar spindle derivative in the spermatid of the rat which he designates as the "idiozome." It also consists of a vacuolar portion which contains a deeply-staining granule which is not a centrosome, just as in *Passalus*.

the centrosome. The conditions here are very much like those in *Passalus* (Figs. 19, 38), but in the latter case the deeply staining body is certainly not a centrosome. The sphere (?) of the spermatid of *Passalus* has no connection with the centrosome. This is in agreement with Montgomery's conclusions in *Euschistus*. According to Munson, the centrosome gives rise to the acrosome of the spermatozoön.

Payne (1917) has approached the subject of spermatid transformation with more or less scepticism as to the generally accepted origins of the structures present here. I am in hearty accord with this point of view, but it seems that Payne has carried matters too far. I agree that to call the structure from which the axial filament grows a centrosome without tracing its history from the second spermatocyte, is highly speculative. But to say, as Payne does, that at a certain stage of the spermatid "there is nothing in the cytoplasm but mitochondria" (p. 309) is equally as dogmatic and unwarranted in light of the observations of many other workers. In *Passalus* there can be no doubt that the centrosome of the spermatid has actually been carried over from the preceding cell-division. Furthermore, in the youngest spermatid there can always be found the refringent cytoplasmic body which is undoubtedly a spindle derivative. In *Gryllotalpa*, Payne finds in the stages succeeding the young spermatids which contain no other cytoplasmic structures but mitochondria, the sudden appearance of two deeply staining bodies, one of which later forms the acrosome, while the other is pushed off into the tail. Since these were not present in the earlier stages, they have apparently arisen "de novo," that is, they are newly differentiated parts of the cytoplasm. One might then expect to find developmental stages of such structures, but Payne does not indicate such. However, it seems to me that the building-up of the spermatozoön from the spermatid is a process involving no differentiation, but a *transformation* of differentiations already present. All the structures needed in the building up of the spermatozoön are at hand, and there is no further elaboration of new ones. Munson (1906, page 96) has clearly expressed a similar view:

"The comparative inertness of the nucleus at the close of the

last maturation division and ever afterward would not justify us in assuming that this is a growth period in the history of the spermatozoön; but rather that it is a transformation period of those organs that are already present and fully-grown in the spermatid stage. This transformation in all the organs of the cell is merely an elongation such as could be brought about, doubtless, by prolonged lateral pressure."

D. General Considerations.

The importance of the mitochondria as bearers of hereditary units rests on their mode of origin and maintenance through the cell cycle, their behavior in fertilization and their rôle in differentiation. If, as has been previously discussed, the view is correct that the mitochondria owe their origin to materials derived from the nucleus or by the activity of the nucleus, then their importance in heredity can only be secondary.

Just how much of the spermatozoön enters the egg is a matter of importance in ascertaining the rôle of the mitochondria. In *Nereis*, Lillie (1912) has found that the middle piece and the tail of the spermatozoön do not enter the egg. On the other hand, Meves (1911) has shown that the entire spermatozoön enters the egg of *Ascaris*, and Van der Stricht (1909) has shown similar results in the bat. It therefore seems impossible to make any generalizations on this subject until more work on the details of fertilization has been done. In the case of *Peripatus*, Montgomery (1912) has shown that the mitochondria are entirely lost in the spermatozoön, being thrown off within certain cytoplasmic lobes. Here, at least, the mitochondria of the spermatozoön can play no part in the transmission of hereditary characters.

The mitochondria have been most exhaustively studied in somatic cells where they are present in a variety of forms. Certain workers (Meves, Duesberg, Hoven and others) have maintained that the mitochondria give rise to myofibrils, neurofibrils and other somatic differentiations; but these views have not been strongly substantiated. The work of Cowdry (1914) is strong evidence that the mitochondria of nerve cells are not transformed into neurofibrils.

The researches on the chemistry of mitochondria are practically all in agreement that they are phospholipins or lecithin-albumins. No one has attempted to show that yolk is a bearer of hereditary units; yet yolk is chemically allied to mitochondria. In fact Fauré-Fremiet has shown that mitochondria actually transform into yolk. The work on the chemistry of mitochondria indicates that they are of great importance in the metabolic activity of cells, but our knowledge of their relation to heredity is negative. To say that the mitochondria are not the bearers of hereditary units is not denying that they may *influence* heredity in some cases, just as we know that heredity can sometimes be influenced by environmental conditions of food, temperature, etc.

One fact which has come clearly to light from this study is that beginning with the spermatogonia and continuing up to the spermatid, there is a progressive elaboration of mitochondria. They are then transformed into a definite structural part of the spermatozoön, the sheath of the axial filament. This progressive increase in the amount of mitochondria seems to indicate that they are differentiation products. Hence if there is any genetic continuity between the mitochondria of successive cell generations, it is only of a limited sort. The conception that the mitochondria present in the somatic cells are the direct descendants of those of the germ cells from which they have arisen, certainly has very little evidence in its favor. It seems more probable that mitochondria are in the nature of cytoplasmic differentiations, akin to metaplasm (yolk, etc.) and without a definite relation to the development of hereditary characters, but with the capabilities of influencing development insofar as they may be related to the metabolic activity of cells. It is possible that in the spermatozoa, the mitochondria merely function as locomotory organs.

Summary.

1. Although mitochondria can not be definitely demonstrated in the primary spermatogonia of *Passalus cornutus*, they are present in the secondary spermatogonia in the form of numerous and diffusely spread granules.

2. The mitochondria increase in number during the growth

period, and in the later stages are found in the form of threads (chondrioconts) which lie in the direction of the chief axis of the cell.

3. During the maturation divisions, the mitochondria envelop the spindle peripherally and are divided by the cell constrictions, so that daughter cells receive approximately equal amounts.

4. The mitochondria of the spermatid form the Nebenkern, which later is pierced by the axial filament. As the latter grows, the Nebenkern elongates, forming a sheath about it.

5. Spindle remains are found forming connections between the spermatogonia. The spindle remains of the last spermatogonial division persist throughout the entire growth period of the spermatocyte.

6. A spindle derivative is found in the spermatid, a portion of which gives rise to the acrosome of the spermatozoön.

7. The centrosome of the second maturation division is carried into the spermatid and gives rise to the axial filament of the spermatozoön. The centrosome becomes so closely associated with the nucleus that there is no well-defined middle-piece in the spermatozoön.

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ABBREVIATIONS.

c., centrosome; *m.*, mitochondria; *N.*, Nebenkern; *s.*, spindle derivative.

All drawings were made with the aid of a camera lucida at table level using a Zeiss 12 ocular and a 2 mm. oil objective. The reproductions have been reduced one-third.

EXPLANATION OF PLATE I.

FIG. 1. Primary spermatogonium. No clear indication of the presence of mitochondria. Note small mass of chromatoid substance in cytoplnsm.

FIG. 2. Anaphase of secondary spermatogonium. Note lightly-staining cytoplasm, well-defined cell-plate, and the tendency of the cell to become round.

FIG. 3. Metaphase plate of ovarian follicle cell, showing thirteen equal pairs of chromosomes.

FIG. 4. Resting stages of secondary spermatogonia. Mitochondria granular and diffusely spread. Note persistence of spindle and cell-plate.

FIG. 5. Degenerating spermatogonium. Mitochondria larger in size; chromatin concentrated in two large karyosomes.

FIG. 6. Pachytene stage of first spermatocyte. Mitochondria still granular, with only a slight indication of threads forming; note denser perinuclear zone and the persistence of the spindle remains of the last spermatogonial division.

FIG. 7. Late prophase of first spermatocyte, Flemming fixation. Nucleus uncut. Mitochondria filar, with their lengths in the direction of the chief cell axis.

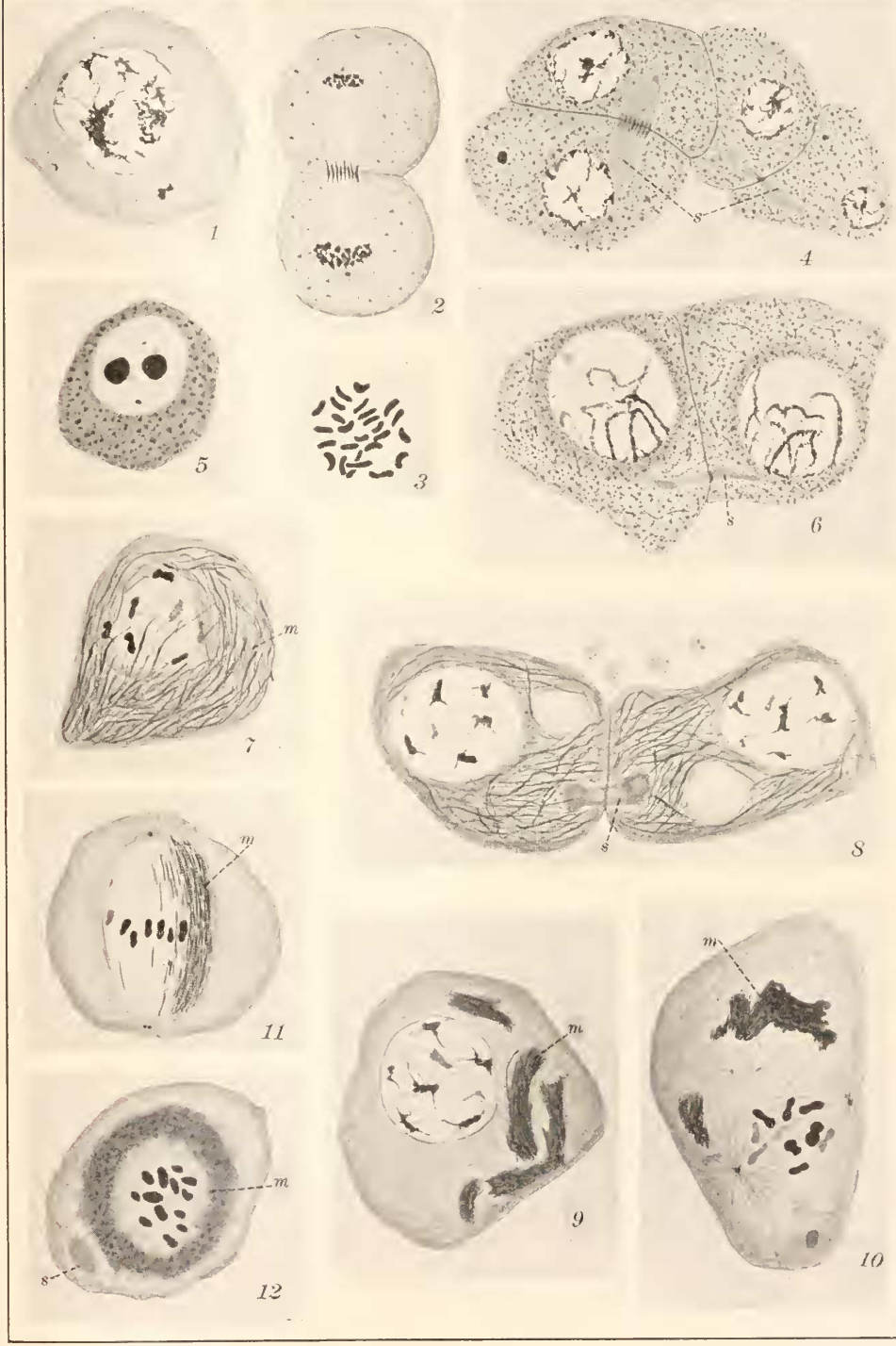
FIG. 8. Similar stage as above, showing remains of spindle still persisting, two large cytoplasmic vacuoles of problematic origin, and small bodies in the cyst cavity which have been budded off from the cytoplasm of the spermatocytes.

FIG. 9. Prophase of first spermatocyte, Hermann fixation; showing agglomeration of the mitochondria.

FIG. 10. Formation of the first maturation spindle; mitochondria at one side.

FIG. 11. Mitochondria beginning to envelop first maturation spindle.

FIG. 12. Cross-section of the metaphase plate of first spermatocyte, showing mitochondria completely surrounding the spindle. Thirteen bivalent chromosomes.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EXPLANATION OF PLATE II.

FIG. 13. Anaphase of first spermatocyte, showing relation of spindle and mitochondria.

FIG. 14. Late anaphase of first spermatocyte. Cell constriction has divided mitochondria so that the daughter cells contain approximately equal amounts.

FIG. 15. Cross-section of first spermatocyte near pole of spindle, showing absence of mitochondria here.

FIG. 16. Second spermatocytes (interkinesis). Mitochondria present in daughter cells in compact masses, also showing the persistence of the spindle and cell-plate.

FIG. 17. Metaphase of second spermatocyte. Mitochondria again surrounding the spindle peripherally.

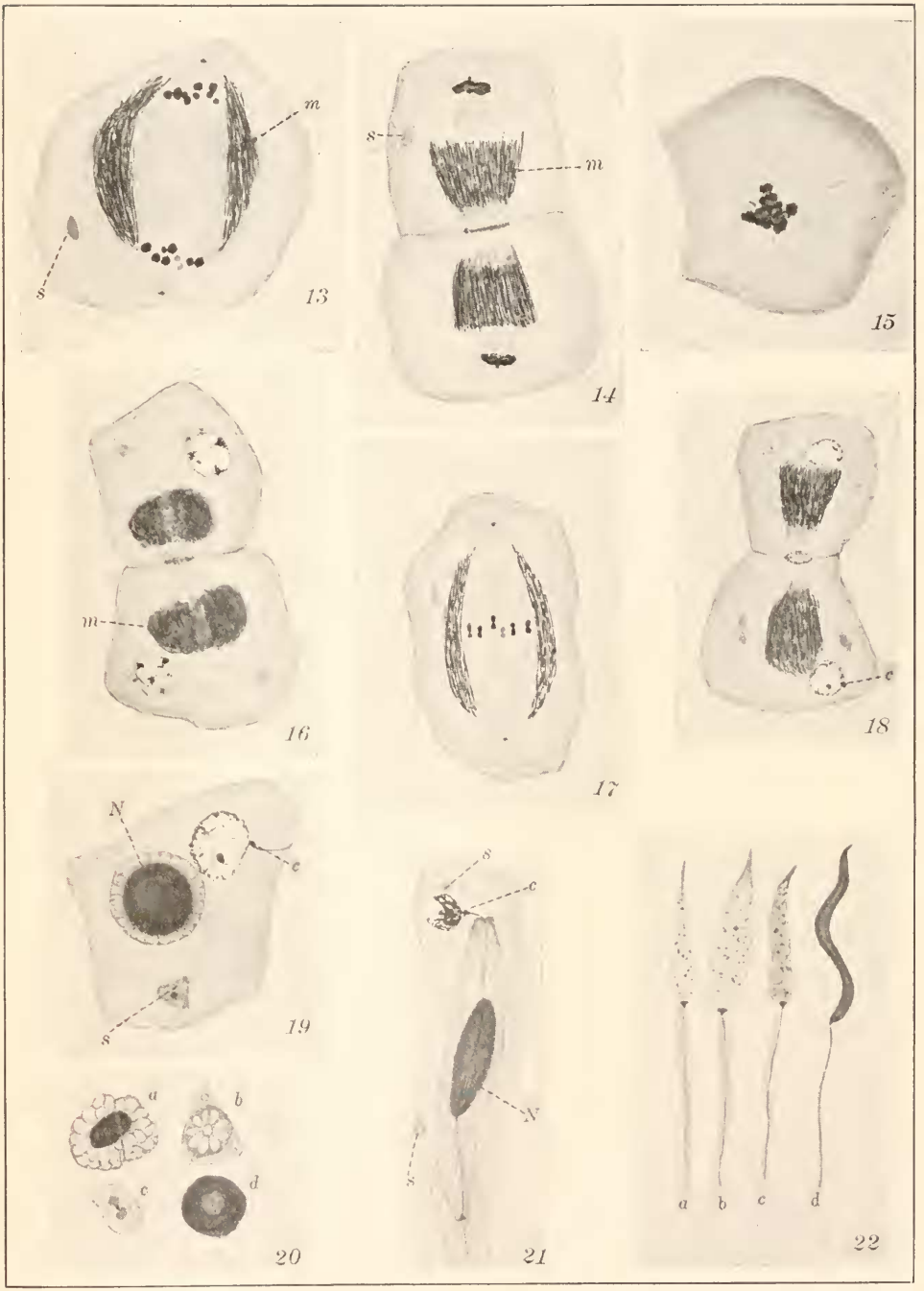
FIG. 18. Telophase of second maturation. Mitochondria again divided by the cell constriction into two equal masses. Note position of the centrosome (*c*) lying on the nuclear membrane.

FIG. 19. Spermatid. Nebenkern (*N*) derived from the mitochondria, with a peripheral vacuolated portion. Persistence of centrosome (*c*) with a precocious growth of the axial filament; spindle derivative (*s*) enclosing a deeply-staining body.

FIG. 20. Changes in the Nebenkern during transformation of spermatid. *c*, cross-section of the tail showing the Nebenkern lying on each side of the axial filament. *d*, Nebenkern with lighter central portion where spindle of previous division had passed through.

FIG. 21. Stage in the transformation of the spermatid. A portion of the spindle derivative (*s*) occupies a position at the head end and a portion passes into the tail. Nebenkern elongating, and the axial filament growing out of the centrosome (*c*) between the two halves of the Nebenkern.

FIG. 22. Later stages in the transformation of the spermatid. *b*, giant spermatid.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EXPLANATION OF PLATE III.

Photomicrographs taken at a magnification of about 1,500 diameters

FIG. 23. Resting secondary spermatogonia, showing diffuse granular mitochondria deeply staining in iron-hæmatoxylin.

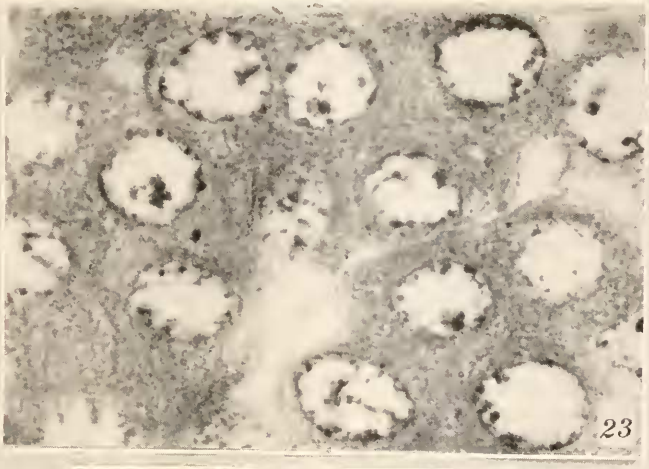
FIG. 24. Telophase of a secondary spermatogonium, showing the deeply staining cell-plate. The rest of the spindle here shows somewhat darker than it really is; it always takes the plasma stain.

FIG. 25. Late prophases of first spermatocyte; Flemming fixation. The mitochondria are filar, with their lengths extended in the direction of the chief axis of the cell; the nuclei here are uncut and somewhat out of focus. Fig. 25*a* is a section at right angles to the chief axis of the cell and shows the mitochondria on end view.

FIG. 26. First spermatocyte from a Hermann fixation. The mitochondria are agglomerated in dense masses (*m*).

FIG. 27. Part of a cyst of first spermatocytes to show the spindle remains (*s*) forming a connection between several cells; parts of it stain deeply with iron-hæmatoxylin.

FIG. 28. First spermatocyte greatly destained to show the cytoplasmic vacuole of unknown significance.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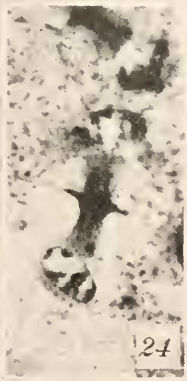
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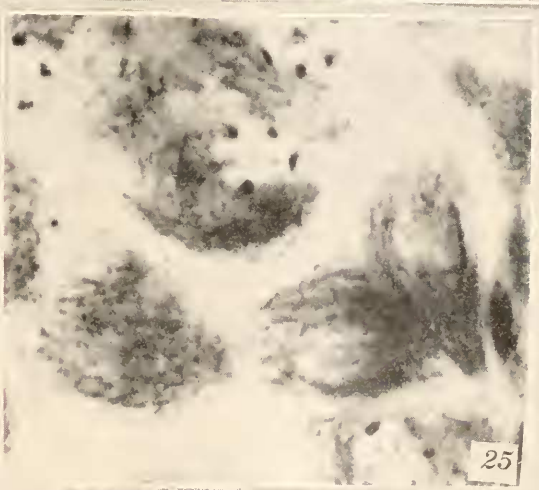
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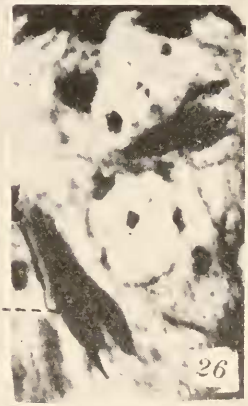

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25 a

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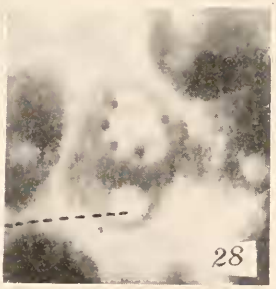

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EXPLANATION OF PLATE IV.

FIG. 29. Formation of first maturation spindle; the mitochondria are at one side and are beginning to envelop the spindle.

FIG. 30. Metaphases of first maturation division; the mitochondria completely surround the spindle.

FIGS. 32, 33, 34. Cross-sections of the first maturation spindle, showing its relation to the mitochondria.

FIG. 35. Late anaphases of the first maturation division; the mitochondria are densely staining and closely applied to the spindle. The mitochondria do not reach to the poles.

FIG. 36. Second spermatocyte; interkinesis. Mitochondria in a dense mass awaiting the formation of the second maturation spindle.

FIG. 37. Second maturation spindle forming with the mitochondria at one side.

FIG. 38. Young spermatid with deeply staining Nebenkern (*N*) lying close to the nucleus, and the spindle derivative (*s*) which contains a deeply staining corpuscle. Centrosome not clearly in focus.

FIG. 39. Later stage in spermatid transformation, showing the elongation of the Nebenkern after the axial filament has grown out.


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